INVESTIGATIONS UNDERTAKEN IN POSSIBLE CASES OF HUMAN PRION DISEASE

INTRODUCTION

Initial diagnosis will depend on the clinical features of the illness that should suggest a prion disease and may point to one particular form of prion disease. It is important to stress that many neurological illnesses may resemble each other in the early stages. Exclusion of other illnesses is critical, especially as some of them are treatable, whereas prion diseases are not.

An absolutely definitive diagnosis of any form of prion disease requires neuropathological examination of brain tissue. This would usually be undertaken at post mortem examination. Rarely, a biopsy of the brain may be taken in life but this is not usually necessary in the investigation of possible cases.

When a diagnosis of prion disease is suspected, investigations are undertaken for two broadly separate reasons. Firstly, investigations are used to exclude other possible diagnoses. Secondly, there are certain investigations which are supportive of the diagnosis of particular prion diseases. These supportive investigations are discussed below. It is important to note that some of these investigations (such as the MRI scan and the cerebrospinal fluid examination) may be undertaken for both reasons.

SPORADIC CJD

There are three investigations which might provide support for a diagnosis of sporadic CJD. These are:

1. The EEG
2. The CSF 14-3-3 estimation
3. The MR scan

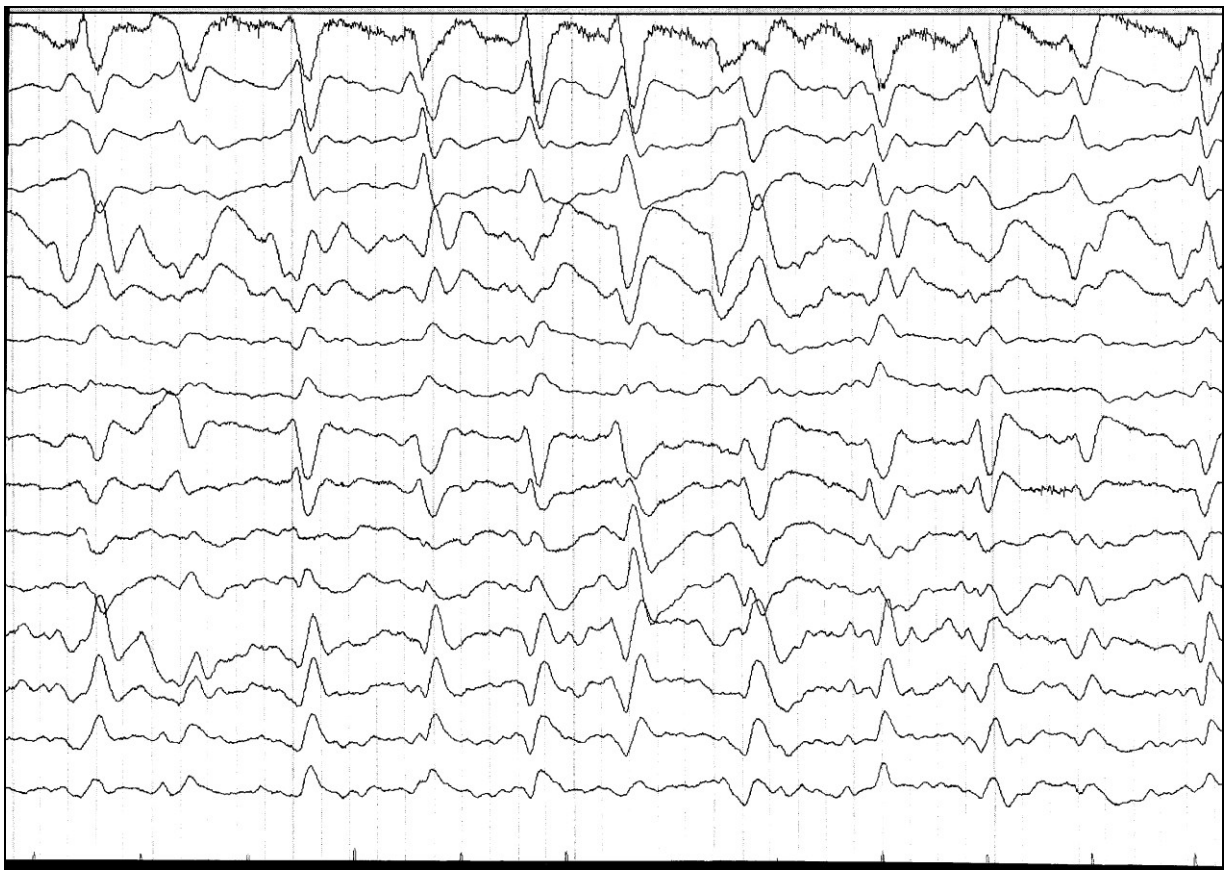
1. THE EEG

In sporadic CJD, the normal electrical rhythms of the EEG are gradually lost. In the majority of cases generalised bi- or triphasic periodic sharp wave complexes appear with a frequency of around 1-2 per second. In an appropriate clinical context, the appearance of this EEG pattern is strongly supportive of a diagnosis of sporadic CJD. However, not all cases show this typical periodic pattern and generalised periodic complexes may appear in other conditions, some of which are listed below. It is difficult to state definitively the frequency of occurrence of the typical periodic pattern in sporadic CJD. This is essentially because the pattern tends to develop throughout the course of the illness and, in some cases, may not appear until very late. Therefore, finding a positive EEG may require repeat studies (possibly weekly) even very late into the illness course and these may not always be undertaken. However, it is probably that about 60 – 80% of cases of sporadic CJD will eventually develop the characteristic periodic picture.

In some cases, the EEG changes may be initially unilateral and indeed the periodic complexes may be unilateral when they first appear.

Note: (This characteristic periodic pattern is less frequently seen in genetic or human growth hormone related cases. It has not been seen in most cases of variant CJD and, if it occurs, it does so very late in the clinical process).

Figure: This is an example EEG tracing showing the characteristic periodic complexes.



Other conditions in which generalised periodic complexes may occur:
Alzheimer's disease
Multiple cerebral abscesses
Metabolic encephalopathy
Certain toxic encephalopathies (eg Lithium)
Anoxic encephalopathy
Progressive multifocal leucoencephalopathy
Lewy body disease

2. CEREBROSPINAL FLUID 14-3-3 ANALYSIS

The routine examination of the cerebrospinal fluid in patients with sporadic CJD is generally unremarkable. The CSF typically contains no inflammatory cells and the presence of a significant pleocytosis should lead to consideration of other diagnoses. The total protein content may be elevated

(usually less than 1 gramme per litre). Oligoclonal bands confined to the CSF have very rarely been described and their significance in relation to sporadic CJD is doubtful.

However, the analysis of CSF for certain brain specific proteins, particularly 14-3-3, may be very useful in diagnosis. 14-3-3 is a normal neuronal protein and maybe released into the CSF in response to a variety of neuronal insults. It is therefore generally a non-specific finding and 14-3-3 analysis cannot be used as a general screening test for sporadic CJD. Other illnesses, which can give a positive 14-3-3 test, include:

- Herpes simplex encephalitis and other viral encephalitides.
- Recent cerebral infarction or haemorrhage.
- Subarachnoid haemorrhage.
- Hypoxic brain damage.
- Glioblastoma
- Carcinomatous meningitis.
- Paraneoplastic encephalopathy.

However, it is usually a straightforward clinical matter to exclude the other possible illnesses which may give rise to an elevated 14-3-3 level. Therefore, in an appropriate clinical context, a positive test is strongly supportive of a diagnosis of sporadic CJD and a negative test is unusual. In the UK, the National Laboratory for 14-3-3 CSF test is in the National CJD Research and Surveillance Unit.

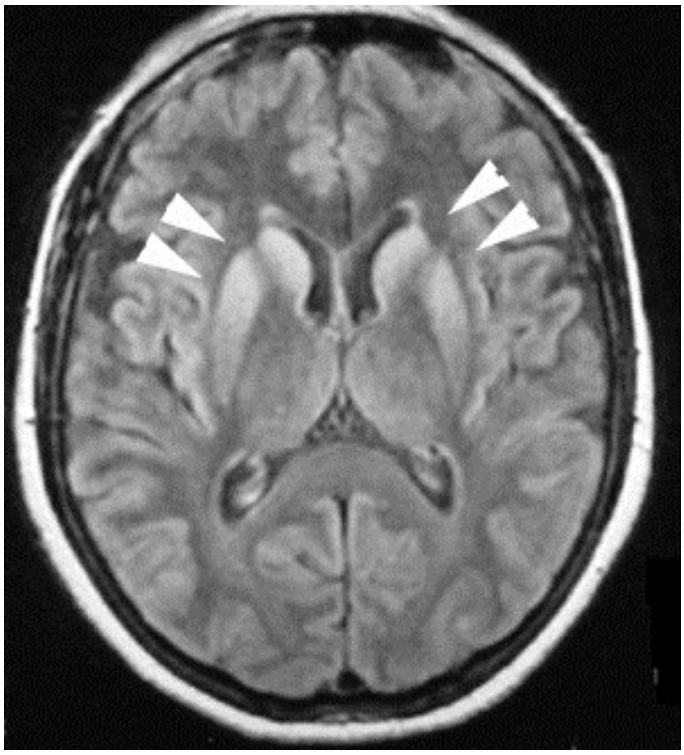
If a case meets the diagnostic criteria for “possible sporadic CJD” and has a duration of less than 2 years then a positive 14-3-3 test allows the case to be classified as “probable sporadic CJD”.

3. MAGNETIC RESONANCE IMAGING

(CT or Computerised Tomography of the brain is usually normal in sporadic CJD although sometimes atrophy may be seen, particularly with long duration illness).

Magnetic Resonance Imaging is generally undertaken to exclude other illnesses. Cerebral atrophy may be seen in cases of sporadic CJD. However, in addition, in a proportion of cases, abnormalities of signal may be seen in the anterior basal ganglia and sometimes in the cortex. These changes may be helpful in supporting a diagnosis of sporadic CJD. Since 2010, MR findings (high signal in caudate/putamen) are included in the accepted clinical diagnostic criteria.

Figure: Transverse FLAIR MRI showing bilateral anterior basal ganglia high signal (arrowheads)



(The EEG may be normal in the earlier stages of variant CJD. Throughout the illness it tends to become non-specifically abnormal. The EEG periodic discharges typical of sporadic CJD are usually not seen although they have been reported twice in the very late stages of vCJD illness).

VARIANT CJD

There are 3 diagnostic tests which provide support for a diagnosis of variant CJD:

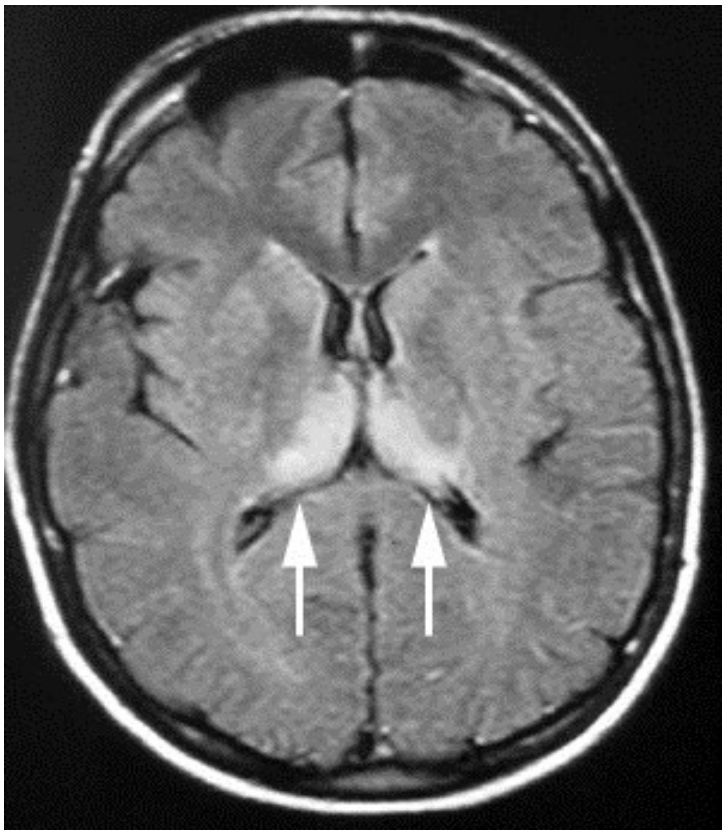
1. The MRI
2. Tonsil biopsy
3. CSF 14-3-3 analysis

1. MAGNETIC RESONANCE IMAGING

The MRI is generally the most useful supportive diagnostic test in variant CJD. It is a relatively non invasive investigation that is generally readily available and, importantly, is undertaken in cases of suspect variant CJD in order to exclude other possible illnesses. However, there is a characteristic abnormality seen in the posterior thalamic region (the so called "pulvinar sign") which is highly sensitive and specific for variant CJD. The pulvinar sign has been found in 90+% of pathologically proven vCJD cases. Present indications are that FLAIR sequences are most likely to show the abnormality. The stage of illness at which the scan becomes positive is uncertain. However, individual patients generally have MR scans at a time when clinicians feel they are appropriate and, as indicated above, partly to exclude other illnesses. It is at this clinically indicated point that positive scans have been found to be positive. In a few patients, the pulvinar sign has been present on MRI within 3 months of symptom onset.

The finding of a positive MRI scan allows a “possible variant CJD” case to be elevated to the category of “probable variant CJD”.

Figure: Transverse FLAIR MRI showing bilateral and symmetrical high signal in the pulvinar nuclei of the thalamus - the 'pulvinar sign' of variant CJD.



2. TONSIL BIOPSY

Unlike other forms of prion disease, variant CJD shows involvement of the lymphoreticular system (lymph nodes, spleen, tonsil and appendix). There is therefore the possibility of finding the disease-related protein in a biopsy of such tissue. Tonsil biopsy has been used as a supportive diagnostic test in variant CJD. However, this does involve a surgical biopsy and its precise role in the investigation of variant CJD is a little uncertain. Its main role is probably to provide support for the diagnosis in cases who have negative MR scans or who have atypical clinical features. A positive tonsil biopsy cannot lead to a diagnosis of definite variant CJD. But, in the correct clinical context, allows a diagnosis of “probable variant CJD”. The surgical biopsy of tonsil must take into account precautions with relation to possible infectivity. The laboratory processing and analysis of tonsil for the presence of the abnormal prion protein is a specialist matter and should be undertaken by laboratories with experience in this.

3. CSF 14-3-3

The general CSF examination is unremarkable, as with sporadic CJD. Again there should be no pleocytosis and the protein level may be non-specifically modestly raised. The 14-3-3 test is not as sensitive in variant CJD as it is in sporadic CJD. A positive result may provide some support for the diagnosis but a negative result by no means excludes the diagnosis. At present, the CSF 14-3-3 test is not incorporated into the routine clinical diagnostic criteria.

GENETIC PRION DISEASE

In genetic prion disease, the definitive test is the analysis of *PRNP* (the prion protein gene) for relevant mutations. This test can be performed on a simple blood sample. Although a family history is usually present in cases of genetic prion disease, sometimes it is not. Therefore, it is generally necessary to undertake genetic testing if one wishes to absolutely exclude the possibility of genetic disease. Aside from analysis for mutations, genetic analysis allows for the determination of the *PRNP* codon 129 genotype (MM, VV or MV). This may have potential relevance in the full characterisation of a case of prion disease. Over 70% of cases of sporadic CJD have the MM genotype but the disease does occur in the other two genotypes. To date, variant CJD (defined as definite or probable on the current agreed diagnostic criteria) has occurred in only the MM genotype. There is one case report of an individual who probably had vCJD (but who did not meet the formal criteria for 'probable vCJD') who was of the *PRNP*-1298 MV genotype.

IATROGENIC CJD

Diagnosis essentially rests on the history of a relevant known risk factor such as treatment with cadaveric derived human growth hormone or the use of human dura mater graft in surgery.

TESTS IN DEVELOPMENT

1. A new CSF test called RT-QUiC has been developed and, to date, is promising as a diagnostic test for sporadic CJD. It is currently being evaluated by the NCJRSU.
    - (McGuire et al. RT-QuIC analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. Annals of Neurology, Accepted Article DOI: 10.1002/ana.23589.)
    - Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. Peden AH, McGuire LI, Appleford NE, Mallinson G, Wilham JM, Orrú CD, Caughey B, Ironside JW, Knight RS, Will RG, Green AJ, Head MW.J Gen Virol. 2012 Feb;93(Pt 2):438-49. Epub 2011 Oct 26

2. A new blood test for variant CJD was reported by the MRC Prion Unit and is currently being evaluated by the National Prion Clinic (Edgeworth et al. Detection of prion infection in variant Creutzfeldt-Jakob disease: a blood-based assay. Lancet 2011; 377:487-493).